Multidimensional Bio-printing Using Hydrogel Suspension

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Abstract

Biological printing (bio-printing) is a process used by tissue engineers to efficiently produce living tissue and organs. Current methods of bio-printing rely on standard three axis 3D printing techniques using free standing printed structures, which limits the ability to print multidimensional soft tissue structures.
In order to overcome this limitation we have developed a system capable of printing into a biocompatible, degradable, hydrogel substrate, which acts as the supportive material for biological inks (bio-inks). We have also created our own hydrogel based bio-ink, capable of solidifying and maintaining its printed structure within the substrate. Our bio-printer also features a new technique for angular printing, allowing any necessary material to be added to the printed structure from any angle, without disturbing the structure.

Introduction

Tissue engineering is a time consuming process involving three main steps: The construction of a supportive scaffold and matrix, the embedding of biological materials or cells, and the maintenance of the biological structures. Bio-printing is a process which utilizes 3D printing methods in order to perform these steps.
There is currently a need in the industry for more advanced bio-printers capable of printing and maintaining full soft tissue structures over the course of several days. We have developed a bio-printer capable of printing standard scaffolding techniques while being able to simultaneously add bio-ink and biomaterials. Our new technique is capable of adding materials from multiple angles, which allows for additions without interfering with features of the primary structure. We have also created an advanced method of hydrogel suspension using a degradable substrate which replaces traditional scaffolding techniques.

Software

Four different levels of software are necessary for breaking down 3D Steriolithography (STL) files for bio-printing.

1) *Slicing*
For our slicing software we have modified the program Slic3r in order to generate a layer by layer, point by point binary file.

2) *Loading*
The sliced version of the file is loaded to the printer's control board via SD card or via a 3D printer loading software called Pruntrface.

3) *Printing Control*
For printing scaffolding structures, we have modified a version of the Arduino Marlin firmware, controlling the speed and step rotation of the printer based on the sliced file.

4) *Secondary Printing Control*
A second version of the Arduino Marlin firmware has been produced to control a robotic arm for use in dual printing for multiple sliced STL files.

Device Design

Bio-printer

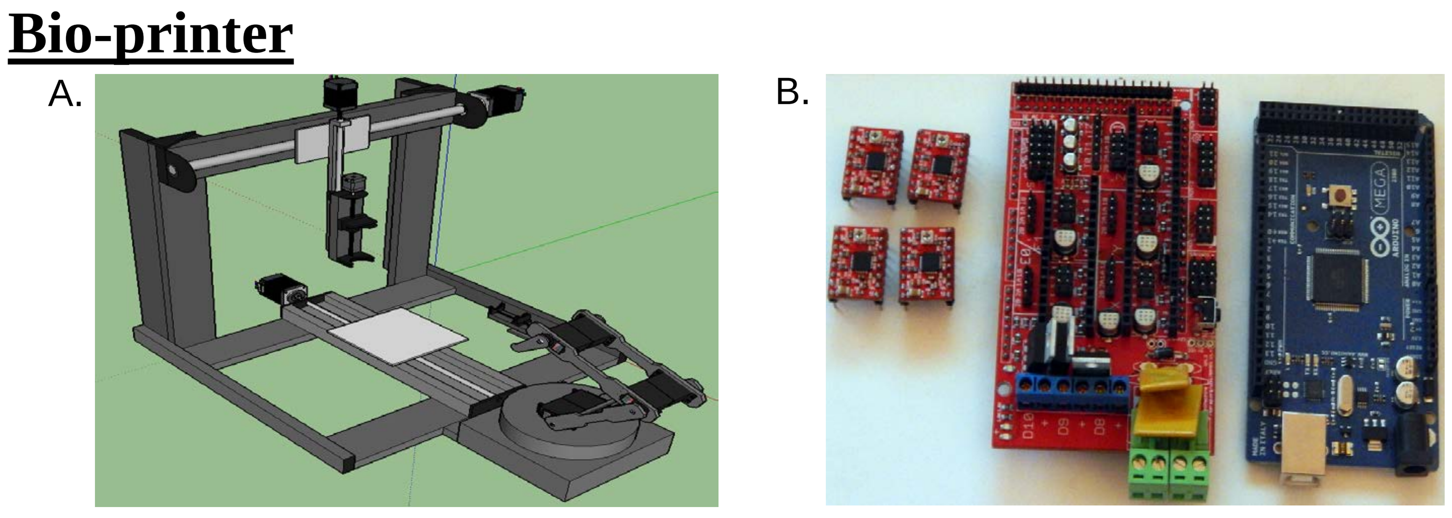

Figure 1. (A) Full bio-printer design. (B) 3D printer control system, featuring (from left to right) stepper motor control chips, RAMPS 1.4 (power control board shield), and Arduino Mega Micro Controller.

The full printer design in *Figure 1A* utilizes a traditional three axis 3D printer containing a modified syringe pump for cold hydrogel extrusion and features a rotating printing platform and four axis robotic arm for the extrusion of the biomaterials. The full system is controlled by two Arduino micro controllers for a dual printing setup.

3 Axis Printer

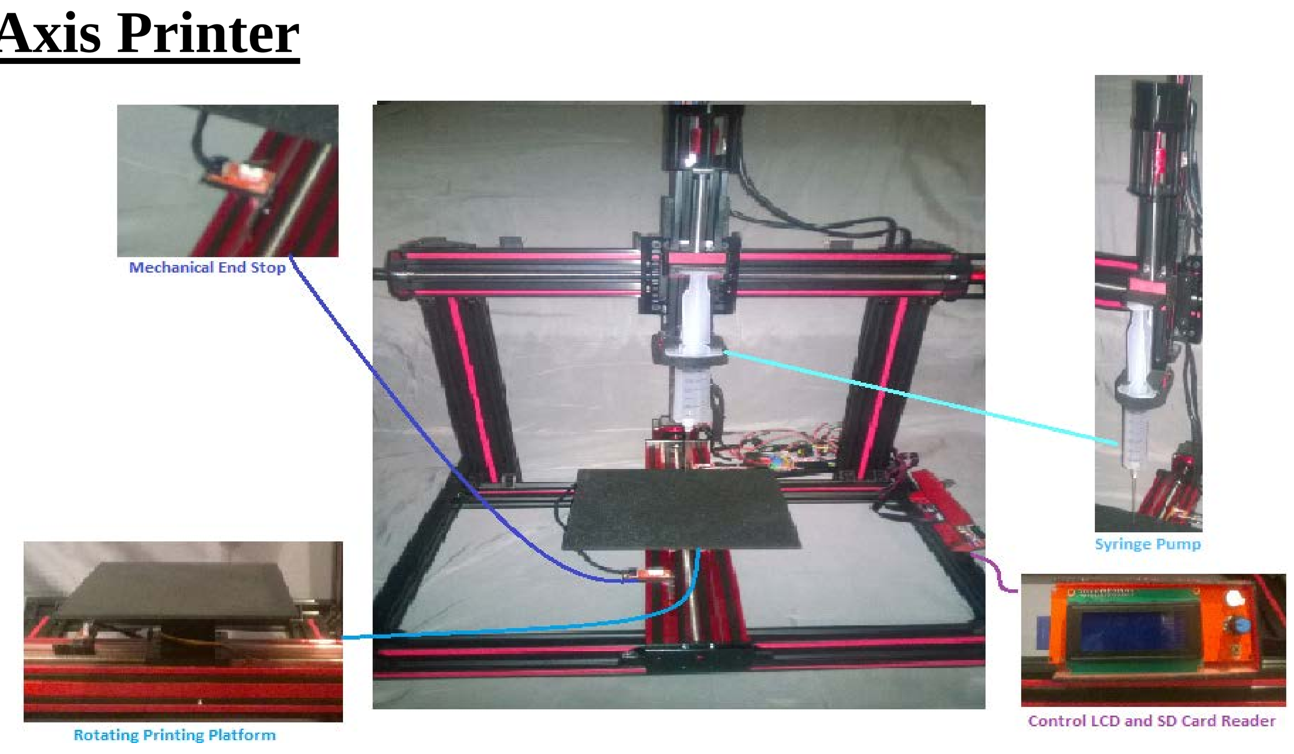

Figure 2. Bio-printer prototype. Features a 60ml syringe pump, 20cmX20cmX20cm print area and mechanical end stop position homing. The syringe features a 30 gauge needle, which when combined with the fine motion stepper motors allow for precision 100µm layering.

4 Axis Robotic Arm

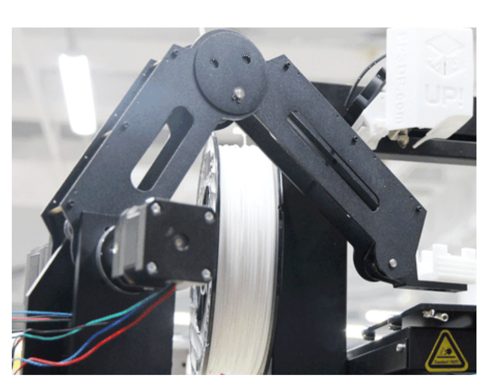

Figure 3. Precision printing arm.

The key innovation in our design is the use of a high precision robotic arm in union with a rotating printing stage. This allows for simultaneous injection of bio-ink, growth factors or hydrogels from 64800 different angels while the main structure is being constructed or after for structural repairs.

Printing Materials

Substrate

We are using granular gel medium made from Carbopol ETD 2020 polymer. This gel is ideal for our technique because it permits repeated retracing of the writing needle due to the local jamming/unjamming transition without a change in composition or material properties. The time scale of this transition is called the thixotropic time; granular hydrogels such as Carbopol are non-thixotropic and ideal because they rapidly stabilize after any abrupt changes in the applied shear stress [2].

Scaffolding and Bio-ink:

Several biocompatible polymer based materials will be used in a specific order to form the scaffold and bio-ink as it is printed inside the substrate:

- ***Synthetic Material:***
  1) PDMS will be used with the addition of a curing agent to stabilize its printed rheology after we inject it inside the substrate.
  2) After printing the PDMS micro structure, it will be allowed to solidify. The substrate will then be washed out and the cellular medium will be prepared and incubated with the resulting scaffold.
- ***Biological Constructs:***
  ECM ink containing a specific protein and a polysaccharide mixture will be added to the gel while maintaining the viability of the printed cells that would have entered the scaffold inside the hydrogel.

Results

Precision Testing

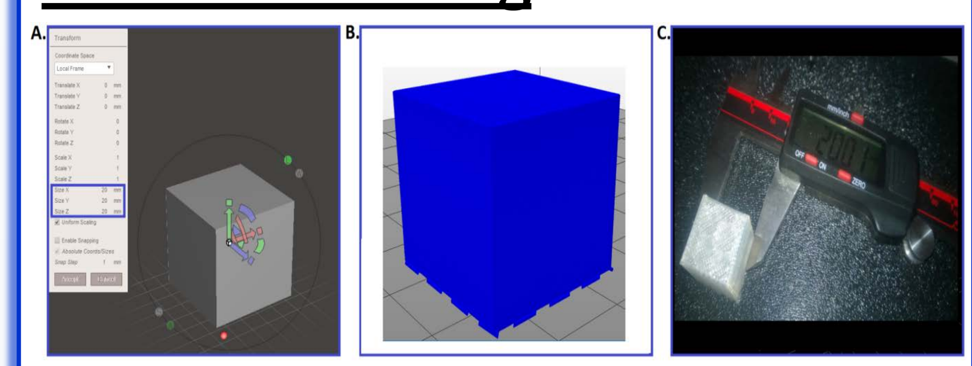

Figure 4. (A) 20mm cube STL file. (B) Slic3r sliced rendering of the original cube STL file. (C) Final print, using heat-extrusion PVA plastic. The final product measured within 20µm of the STL design in all directions.

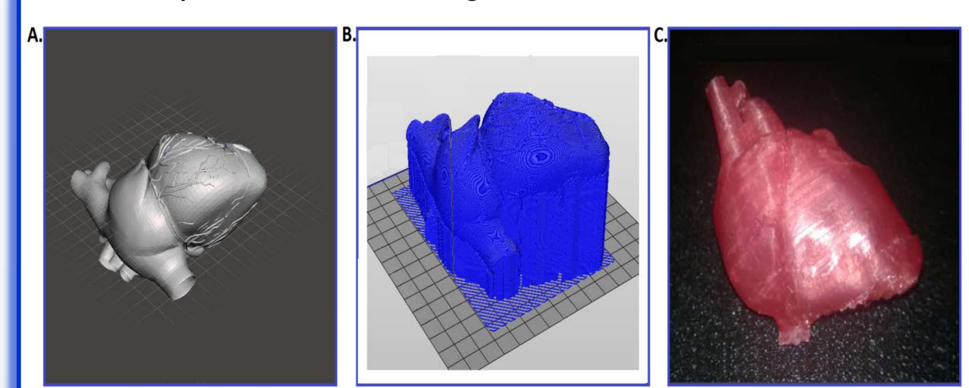

Figure 5. (A) Human heart STL file. (B) Slic3r sliced rendering of the original heart STL file. (C) Final print, using eosin stained quick solidifying ABS.

Materials Testing

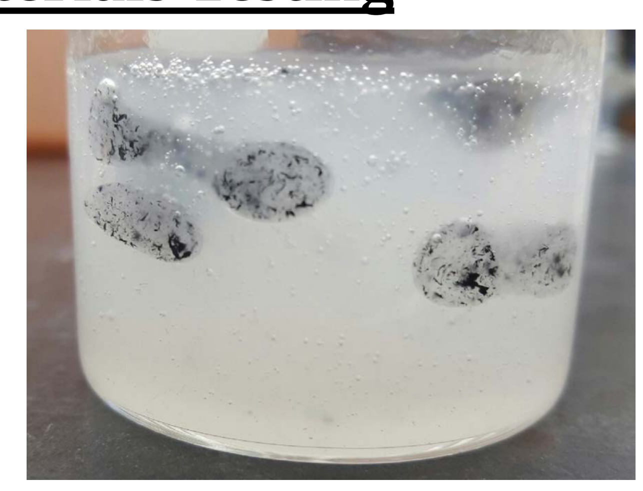

Figure 6. Suspended graphene infused PDMS tubes containing 10% curing agent, held in position within 100ml of Carbopol ETD 2020 hydrogel.

Conclusion

We were able to manufacture a 3D bio-printer capable of printing synthetic prototypes of biological structures. In the future, we plan to expand the testing capabilities and methods to produce more elaborate and complex microstructural scaffolds; such as highly fragile vascular networks for the tissue engineering industry. Furthermore, we plan to use different biocompatible materials that can be directly embedded making a more favorable media for the cellularization of the fabricated scaffolds.

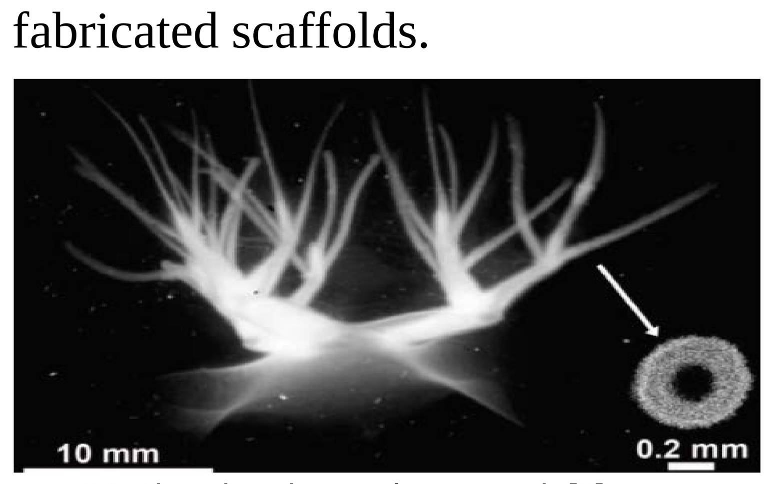

Figure 7. 3D Bio-printed vascular network [2]

References

[1] Hinton, Thomas J. et al. "Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels" Science Advances 2015; 1(9), e1500758 doi10.1126/sciadv.1500758

[2] Bhattacharjee T, Zehnder SM, Rowe KG, et al. "Writing in granular gel medium" Science Advances. 2015; 1(8):e1500655. doi10.1126/sciadv.1500655

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